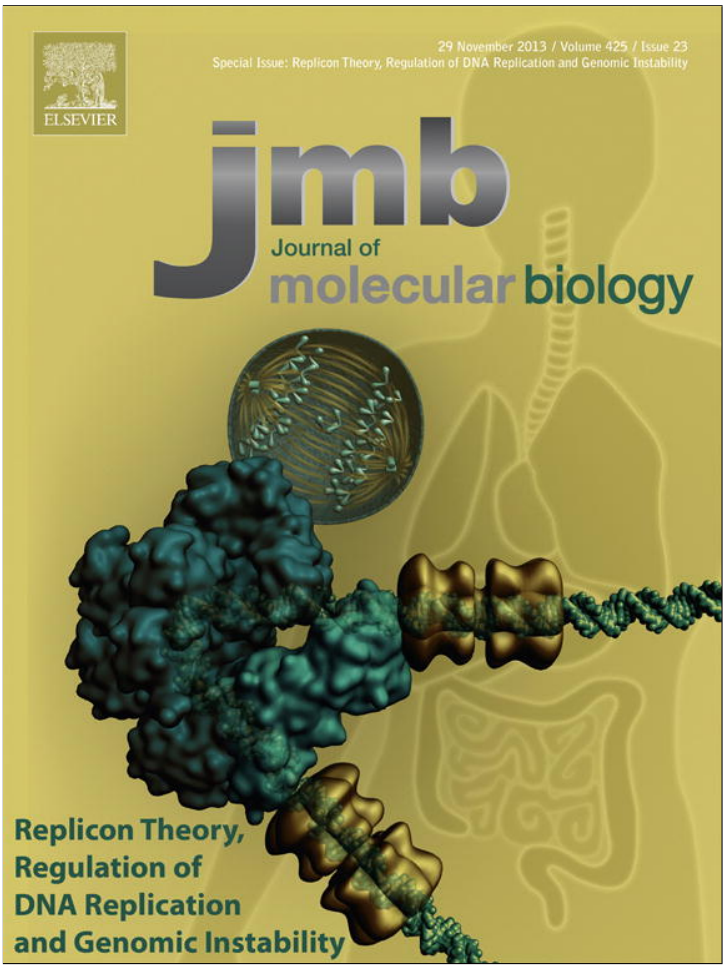

jmb Perspective

A Personal Reflection on the Replicon Theory: From R1 Plasmid to Replication Timing Regulation in Human Cells

Hisao Masai

Department of Genome Medicine, *Tokyo Metropolitan Institute of Medical Science, 2-1-6 Kamkitazawa, Setagaya-ku, Tokyo 156-8506, Japan*

Correspondence to Hisao Masai: *masai-hs@igakuken.or.jp*
http://dx.doi.org/10.1016/j.jmb.2013.03.039
Edited by M. Debatisse-Buttin

Abstract

Fifty years after the Replicon Theory was originally presented, detailed mechanistic insight into prokaryotic replicons has been obtained and rapid progress is being made to elucidate the more complex regulatory mechanisms of replicon regulation in eukaryotic cells. Here, I present my personal perspectives on how studies of model replicons have contributed to our understanding of the basic mechanisms of DNA replication as well as the evolution of replication regulation in human cells. I will also discuss how replication regulation contributes to the stable maintenance of the genome and how disruption of replication regulation leads to human diseases.

Introduction

The beauty of the Replicon Theory is its amazing simplicity. Only two loci, SGI (structural gene for initiator) and replicator, were hypothesized as critical elements for DNA replication and it was proposed that the initiator positively regulates initiation at the replicator.[1] Another important aspect of the Replicon Theory was the proposal that membranes play crucial roles in regulation of initiation of DNA replication. The latter issue, which has not yet been completely resolved, will not be dealt with in this article but will be briefly touched on later. Discovery of *oriC* and *dnaA* in the following years[2,3] upheld the model as an accurate description of replication in bacteria, although biochemical evidence had to wait for another decade or more.[4] Although it was evident from earlier studies that eukaryotic chromosomes contain multiple replicators, the nature of the initiator remained elusive until Orc was discovered in yeast.[5] Now, the basic concept of the Replicon Theory has proven to apply for both prokaryotic and eukaryotic replicons.[6]

As in Arthur Kornberg's famous 10 commandments (VI. Depend on Viruses to Open Windows),[7] viruses played crucial roles in elucidating mechanisms of DNA replication. Resolution and reconstitution studies saw first triumph when replication of small single-stranded virus DNAs was reconstituted with purified enzymes.[8] This approach was quickly adopted for studies of animal virus replication, which provided important insight into the DNA chain elongation stages of eukaryotic DNA replication.[9]

The enzymatic studies on initiation at the chromosome replicator had to wait for development of a soluble enzyme system, which was made possible only after careful fractionation of crude extracts and inclusion of a polymer (polyethylene glycol or polyvinyl alcohol), which presumably caused a molecular crowding effect.[10] Once a system was available, the rest followed rapidly. In only a few years, initiation at *oriC* was reconstituted with purified proteins,[11] and the nature of dnaA (initiator)–*oriC* (replicator) interaction was resolved.[4,12]

R1 Plasmid as a Model for Regulation of DNA Replication

The presence of the bacterial chromosome replicator, *oriC*, was suggested in the 1960s by marker transfer experiments in *Bacillus subtilis* and identified genetically in *Escherichia coli* in the early 70s.[3,13] However, the molecular details were unknown until it was cloned as an autonomously replicating sequence supporting the episomal maintenance of

the linked antibiotic resistance gene.[14] *oriC* was also isolated as a novel F′ DNA segment capable of replicating on Hfr or as a DNA segment capable of bypassing lambda immunity.[15–17]

Plasmids are generally circular double-stranded DNA stably maintained in the cytoplasm of bacterial cells independent of the host chromosome. Plasmids can confer drug resistance or fertility to host cells but are normally non-essential for host maintenance. It has become obvious that each plasmid is an independent replicon composed of its own initiator and replicator. Since replication of most plasmid DNA depends almost entirely on the host replication machinery, plasmids have served as excellent model systems for host chromosome replication.[18]

The R1 plasmid, belonging to the IncFII incompatibility group, encodes multiple drug resistance genes and is strictly maintained at one to two copies per host cell.[19] The minimum segment required for replication contains a gene called RepA, which was likely to be an initiator for R1 plasmid replication.[20] We first used an *in vitro* replication system that depends on *de novo* protein synthesis.[21] The replicator, *oriR*, was mapped using the *in vitro* trans-complementation assay.[22] The purified RepA protein can bind specifically to the sequences within *oriR* and induces partial melting (or

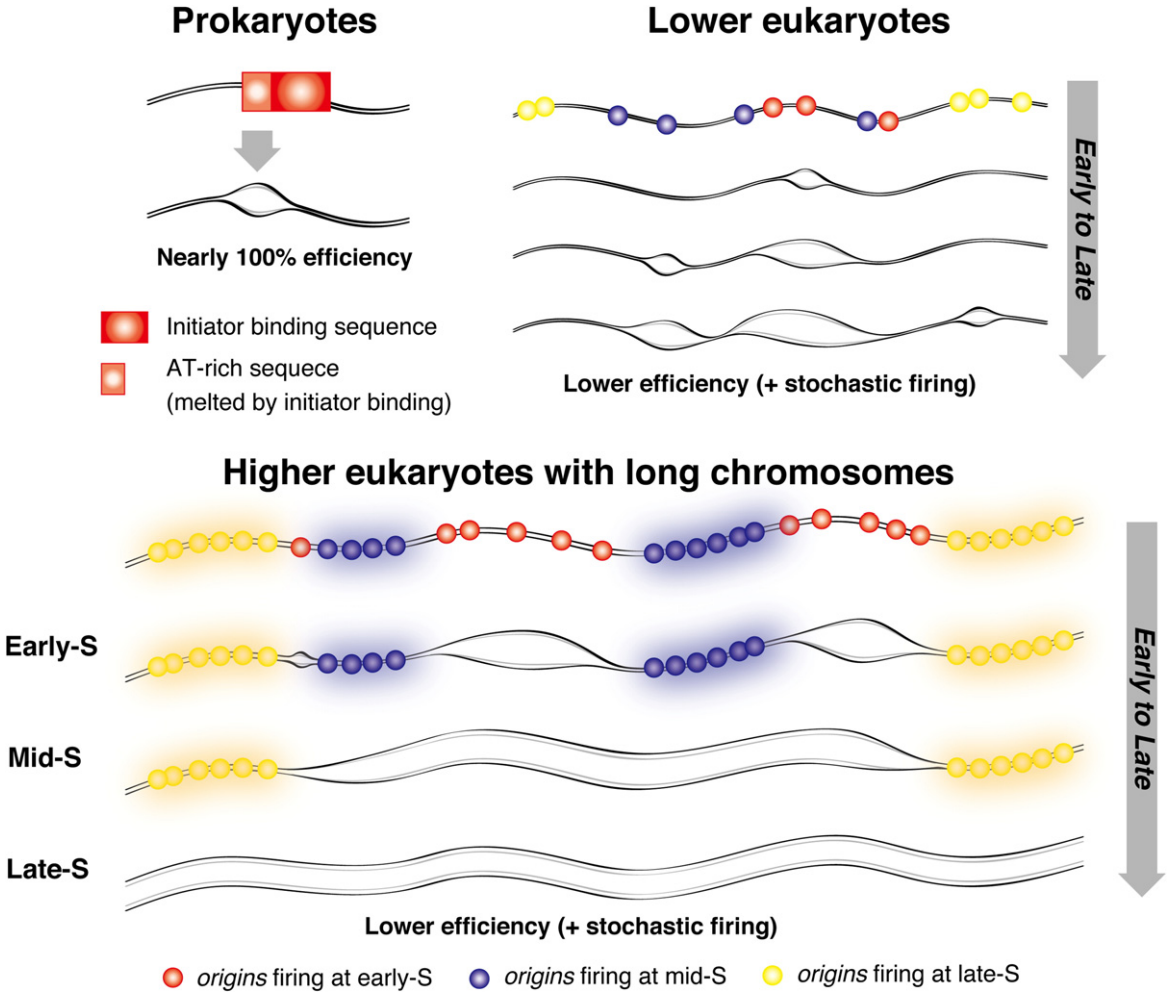

Fig. 1. Initiation at the replicators of various organisms. (Upper left) In prokaryotes, replicators (*origin*) are composed of the initiator binding sequence and the adjacent AT-rich sequences that are melted upon initiator binding. In most cases, there is only one replicator on each genome, and efficiency of initiation is nearly 100%. (Upper right) In lower eukaryotes (e.g., yeast), *origins* fire at different timing. Firing at each *origin* can be stochastic. The efficiency of firing at each *origin* is low and which *origin* is fired may vary from one cell to another. (Lower) In higher eukaryotes with longer chromosomes, the neighboring *origins* (shown by the same colors) are activated at a similar timing, forming replication-timing domains. Mid-S replication domains (containing blue *origins*) are sequestered from activation in early S and cannot be fired until mid-S. This temporal inhibition is mediated by the Rif1 protein. Late-S replication domain (containing yellow *origins*; mainly composed of the heterochromatin region) may be similarly sequestered until late-S, but what mediates the suppression is not known. The efficiency of firing of each *origin* is low and which *origins* in the same replication domain are activated may be under stochastic regulation.

Table 1. Once and only once replication of plasmid R1 and eukaryotic chromosomes

	R1 plasmid	Eukaryotic chromosome
Once and only once replication	During host cell cycle	During S phase
Proteins	RepA protein	Licensing factors (Cdt1-Mcm)
Unique initiation	*De novo* synthesis prior to initiation and coupling with translation	Licensing established only prior to each initiation
Inhibition of re-replication	Inactivation after action at *oriR* (mechanism unknown)	Unavailable after action (degradation, exclusion from nuclei, action of an inhibitor, etc.)

The mechanisms for once and only once replication of plasmid R1 and eukaryotic chromosomes are compared. (R1 replication can occur more than once during a host cell cycle, and thus it is not "once and only once" in a strict sense. However, we would like to emphasize the conceptual similarity between the two regulatory systems.)

structural change) of duplex DNA at the AT-rich segment within *oriR*.[23] Similar studies were conducted at the replicators of various plasmid replicons as well as at *oriC*, leading to the unified view on the prokaryotic mode of initiator–replicator interaction and function[24] (Fig. 1).

During the course of our study, it became evident to us that RepA protein needs to be synthesized each time before initiation. Furthermore, it can activate only the origin present on the same template from which it was synthesized.[22] It is inactivated each time after it triggers initiation at the replicator. Further analyses showed the requirement of the *CIS* segment downstream of the *repA* gene carrying a *rho*-dependent transcriptional terminator for the *cis*-action.[25] The RepA trapping model was proposed in which the nascent RepA protein, synthesized on the paused mRNA, is first trapped at the entry sites present at the C-terminal coding segment of *repA*, followed by its one-dimensional diffusion to locate *oriR*.[25,26] After RepA fires initiation at *oriR*, it is inactivated or made unavailable for reuse by some unknown mechanism. The *cis*-action of the RepA protein is the central system to ensure once and only once replication of the R1 plasmid, reminiscent of the origin licensing system in eukaryotes[27] (Table 1). Multiple mechanisms have been shown to operate to inhibit re-replication at *oriC*[28] as well as that of eukaryotic chromosomes,[29] pointing to the critical importance of the system to ensure "only once" replication in all organisms.

Although my comments were focused on plasmid R1, I should mention that studies of replication on other plasmids (ColE1, R6K, λdv, F, pSC101, RK2, etc.) played very important and pioneering roles in dissecting the structure of replicators, initiator–replicator interactions, events during initiation, and molecular mechanisms of regulation.[30–35]

Replicator–Initiator Interactions in Prokaryotes and Eukaryotes

As discussed in the previous section, prokaryotic replicator–initiator interactions are highly replicon specific, meaning each initiator will only interact with its appropriate replicator. In addition, the replicator–initiator interaction is sequence specific. Generally, one base substitution abrogates the interaction, but once the initiator binds to the replicator and an initiation complex is assembled, initiation efficiency is almost 100%. In contrast, recognition of eukaryotic replicators by the eukaryotic initiator, Orc, appears to be much less specific. Although Orc in lower eukaryotes (e.g., budding yeast) was reported to bind to the cognate replicator in a sequence-specific manner,[5] Orc from human binds to DNA in a sequence-independent manner *in vitro*.[36] Whether Orc binds to chromosomes with some preference is still under debate.[37] Genome-wide mapping of Orc and Mcm would provide some insight into this subject.[38] Further contrasting prokaryotic replicons, efficiency of firing at each replicator is fairly low in eukaryotes, perhaps even occurring in a stochastic manner to a certain extent[39–41] (Fig. 1).

DnaA protein can induce localized melting (or structural changes detectable with single-stranded DNA-specific probes) within the *oriC*,[42] and other prokaryotic initiators can exert similar effects within the cognate replicators. In archaea, whose replication machinery shares more similarity with that of eukaryotes than the prokaryotic counterpart, Orc binds specifically to the repeat sequences present in the origins and induce distortion of duplex DNA, which leads to localized unwinding at DUE (DNA unwinding element[43–45]). However, similar effects have not been detected with Orc or pre-RC bound at eukaryotic origins, although RPA binding can be detected, which indirectly shows the generation of single-stranded DNA.[46] Mcm has been shown to bind to origins as a double hexamer encircling the duplex DNA,[47,48] which is converted to two CMG helicase complexes[49] that encircle the single-stranded leading-strand template DNAs at each replication fork.[50] A recently developed *in vitro* replication system dependent on a pre-RC generated at a specific origin sequence[51] holds great promise to clarify the structural change of template DNA during initiation steps.

Regulation of Origin Selection and Timing of the Firing

In eukaryotes, DNA replication starts at multiple sites along the chromosomes. The numbers of the

replicators (defined as the locations of pre-RC assembly) exceed those of actual initiation events occurring within S phase. Many replicators are dormant during normal S phase and may be used only in an emergency when normally used replicators are unavailable.[52,53] Even the active replicators are used only in a fraction of cells, a fraction of time, reflecting the inefficiency of firing.[40,54] Replicator choice and timing of their firing are coordinated with S phase progression and appear to be predetermined in the population of cells (Fig. 1). How do cells determine the program of DNA replication?

Replication stress activates a checkpoint that suppresses the firing of late origins and/or slows down ongoing replication fork through the actions of checkpoint sensor and effector kinases. Studies in yeasts showed that suppression of the replication checkpoint (by mutation in Mec1 or Rad53 kinase) leads to the precocious firing of otherwise late-firing or dormant replicators.[55,56] This could be due to loss of inhibitory signals that suppress the firing of these inactive origins. Some of the targets of this checkpoint may be Sld3 and Dbf4, which are crucial for conversion of the pre-RC into an active helicase complex at the fork.[57,58] It was also proposed that the more efficient recruitment of pre-RC components may facilitate earlier firing of replicators.[59] It was reported in yeasts that replicators compete for limiting replication factors including Cdc7-Dbf4 kinase and Sld3 and overexpression of these factors promotes firing of late/dormant replicators in early S phase.[60,61]

Other classes of proteins that affect the replication program are modifiers of chromatin including histone deacetylase and methylase. Among them, the Rpd3 histone deacetylase affects the timing of origin firing genome-wide, since *rpd3*Δ mutation advances the firing of many late/dormant replicators.[62] This suggests that chromatin structure affects replication initiation. HBO1 histone acetylase facilitates pre-RC formation through interaction with Cdt1,[63] thus indirectly affecting firing efficiency. Recently, Forkhead transcription factors, Fkh1 and Fkh2, were shown to confer early, efficient firing of sets of origins by inducing clustering of the origins through its interaction with Orc in budding yeast. The origin clustering would increase the effective concentrations of initiation factors around origins, facilitating initiation.[64] This appears to be a novel mechanism for marking early-firing origins.

Studies in yeast have shown that some sequences associated with late-firing origins can enforce late replication on otherwise early-firing origins.[65] Further dissection showed that telomere-like repeats are responsible for this suppression. Indeed, deletion of Taz1, a telomere binding factor, caused the subtelomere regions as well as some arm segments to replicate early, presumably by binding to the telomere-like sequences.[66]

Cdc7 kinase plays a crucial role for initiation of DNA replication at each replicator by phosphorylating the Mcm subunit of the pre-RC.[67,68] Although essential for normal growth, Cdc7 function could be bypassed in budding yeast by mutations in Mcm; they are *mcm5* P83L mutation (*bob1*)[69,70] and the internal deletion at the N-terminal segment of *mcm4*.[71] These presumably change the conformation of the Mcm complex, relieving the requirement of Cdc7 for initiation. Although similar *mcm* mutants that could bypass Cdc7 in other species have not been reported, we found that checkpoint mutations including *cds1* and *mrc1* could restore the growth of *hsk1*Δ, the fission yeast homologue of Cdc7.[72,73] Screening of bypass mutants for *hsk1*Δ led to isolation of *rif1*Δ, which very efficiently rescued growth of *hsk1*Δ cells.[74] Rif1, originally identified as a telomere binding protein in budding yeast, binds to telomere/subtelomere regions in fission yeast and suppresses the early replication of these segments. We found that the replication timing of arm segments is also dramatically affected in *rif1*Δ cells. Importantly, not only late/dormant origins were prematurely activated, but also early-firing origins were suppressed in *rif1*Δ, causing the firing of most origins to shift toward mid-S.[74]

In higher eukaryotes, it has been known that clusters of replicators are fired at similar timing during S phase[75,76] (Fig. 1). Recent genome-wide studies revealed the presence of "replication-timing domains" that are replicated at specific timing of S phase.[77,78] Earlier studies have shown that replication timing of metazoan chromosomes is determined at a timepoint in early G1 phase termed TDP (timing decision point),[79] which was shown to be associated with chromatin repositioning. We and others found that mammalian Rif1 protein plays a major role in setting up the replication-timing domains.[80,81] It plays an essential role in generating the mid-S replication domains by binding to nuclease-resistant nuclear structures at late M/early G1 and presumably facilitating the formation of chromatin loop structures (Rif1 loop; see graphical abstract[80]). The origins present in these mid-S replication domains are somehow sequestered from firing until mid-S phase (Fig. 1 and graphical abstract). Rif1 forms nuclear foci, enriched at the nuclear membrane and the nucleolar peripheries, coinciding with the locations of mid-S replication foci.[81,82] Rif1 may facilitate association of chromatin with the nuclear membrane to enforce mid-to-late replication.[82] In fact, budding yeast Rif1 protein localizes to the nuclear periphery dependent on a palmitoyltransferase,[83] suggesting that Rif1 may be anchored to nuclear membrane through lipid modification. Thus, critical roles of membranes in regulation of DNA replication could be conserved from prokaryotes to eukaryotes.

It is interesting to note that two critical events for DNA replication in S phase, namely, the *origin*

licensing (helicase loading) and *mid-S replication domain licensing* (generation of special replication domains for mid-S), independently occur at late M/early G1 (see graphical abstract).

"Strict" and "Soft" Regulations of Eukaryotic DNA Replication

As discussed above, the inhibition of re-replication is strictly enforced in every species and aberrancy in this regulation can lead to increased genome instability and ultimately to various diseases, most notably cancer/tumor formation.[84–86] In contrast to this strict regulation, once cells enter S phase, regulation at the level of active origin selection or firing time appears to be rather flexible, representing the "soft" regulation of eukaryotic DNA replication. Indeed, origin firing patterns can be simulated by a stochastic firing model if one accepts two assumptions: the difference in firing probability between replicators and the increase in firing probability during S phase.[87] The firing probability of each replicator may be affected by many genetic, epigenetic, and physiological parameters.

Although replication-timing regulation is largely lost in *rif1*Δ fission yeast cells, S phase progression is almost normal and the mutant is resistant to various genotoxic stresses including HU and MMS.[74] Although Chk1 is slightly activated,[80,81] Rif1 depletion in HeLa cells similarly does not cause much effect on S phase progression in spite of major structural changes to replication-timing domains. These observations suggest that cells are quite tolerant to perturbation of replication timing. They are programmed to complete the S phase once it initiates the process of DNA replication, since obviously the abortion of DNA replication in the middle of the process would be devastating to cells' fate. *hsk1*Δ cells are not viable at 30 °C or below, but are viable at 37 °C, at which some dormant origins are fired. These results indicate that the origin firing program is plastic and could change under various physiological conditions.[73]

Replication Fork Arrest and Maintenance of Genome Stability

Replication fork progression can be disturbed by many internal or external causes including reduced level of nucleotide supply, proteins bound to the template, unusual DNA structures, agents influencing the replication machinery, DNA damage, and so on. Stalled replication forks need to be detected and protected to avoid disintegration of the fork structure that could lead to DNA lesions and eventually to unwanted rearrangement or mutagenesis. In prokaryotes, there are only two replication forks on a single replicating chromosome, and stalling of one fork would leave a large unreplicated segment. Thus, in the event of replication fork stalling, restoration of the active fork is essential.

Resolution and reconstitution studies on φX174 phage DNA in the 1970s led to the discovery of a set of genetically unidentified proteins.[88] Proteins i and n′ were among them, and their identities had not been discovered until the genes for these proteins were cloned. Protein i is encoded by dnaT[89] and n′ was a novel gene designated priA.[90,91] The physiological role of the φX174-type primosome, a protein complex capable of duplex unwinding and primer RNA synthesis for lagging strand, was unclear but identification of protein i as DnaT (essential for inducible stable DNA replication, an *oriC*- and dnaA-independent mode of DNA replication of the *E. coli* chromosome induced by DNA damages) suggested a possibility that it plays a role in the alternative mode of chromosome replication. Indeed, PriA is also essential for the *oriC*-independent mode of chromosome replication.[92] However, severe growth defects of *priA*Δ cells suggested essential roles of PriA during the normal course of replication.[93–95] Purified PriA protein bound to D-loop-like structures or arrested fork-like structures *in vitro*.[96] *priA*Δ cells are extremely sensitive to fork stalling agents, and thus, it was proposed that PriA recognizes the stalled replication fork and promotes assembly of "primosome" for fork restart.[95,97] In fact, the fork restart process has been reconstituted with purified proteins.[98]

Detailed analyses of how PriA interacts with the stalled fork revealed the presence of a pocket structure (TT-pocket; *T*hree-prime *T*erminus recognition pocket) that accommodates the 3′-terminus of DNA.[99,100] PriA specifically recognizes the 3′-terminus of the nascent leading strand at the stalled replication fork, stabilizes it, and triggers reassembly of the active replication fork.[101]

On eukaryotic chromosomes, chances of fork stall are larger due to the presence of multiple replication forks at any given time during S phase. Therefore, the stalled fork needs to be swiftly dealt with to prevent fork collapse or aberrant fork structures,[102] which would be most detrimental to the cell survival and maintenance of genome stability. Thus, eukaryotic replication machinery is equipped with many auxiliary factors that facilitate the stabilization and protection of the fork in cases where fork progression is blocked.[103] These factors not only protect the fork but also send out signals to promote any necessary cell cycle arrest or DNA damage repair. The fork could be restarted at the stalled site, but restart may not be as crucial as in prokaryotes since nearby dormant origins can be activated to finish any unreplicated segments.[104]

In carcinogenesis, initial oncogenic stress may cause reduction in the level of cellular nucleotide precursors, leading to the blockage of replication fork progression.[105–107] This can be detected by the

appearance of DNA damage foci. Cells in this precancerous state may be converted to a malignant state if the DNA damage response system fails and large-scale genomic instability is induced.[106] More recently, it was shown that error-prone DNA replication is induced upon recombination-dependent restart from the stalled fork in fission yeast, directly linking the fork stall to increased genomic instability.[108,109] These reports underpin the importance of the cellular response to stalled replication forks. Indeed, mutations in various checkpoint/replication/repair factors have been reported to cause chromosome instability syndrome, most of which are cancer predisposed.[110,111]

Concluding Remarks

It is amazing that one simple theory provided guidance for the research that followed it for 50 years, which has now proven that the basic concept of the theory is correct from *E. coli* to humans. The structures of bacterial replicators have long been dissected,[112] and those of lower eukaryotes have been analyzed in depth.[113] Until now, however, the structures of mammalian replicators have been rather elusive,[114] due to the lack of a consensus sequence and to the lack of a convenient system to assay origin function. Recent technological advances for mapping replication origins or initiator binding sites genome-wide have made it possible to catalogue all the potential replicators and deduce some common structures[38,115,116]. It was indeed suggested that replicators may be generated at any nucleosome-free intergenic segment to which Orc has high affinity.[117] Future studies will uncover the nature of the determinants for the replicators in higher eukaryotes.

Initiators have been identified in both prokaryotes and eukaryotes. The site-specific binding of DnaA and Orc determines where replication is initiated and their functions are regulated by bound nucleotides.[5,118] High specificity of prokaryotic replicator–initiator interactions is associated with highly efficient initiation, while the specificity generally decreases with the increase of genome complexity and this probably contributes to more plastic and adaptive regulation of initiation events in higher eukaryotes. Attempt to determine the genome-wide distribution of Orc binding sites could provide important clues as to the determinants for recognition by Orc in mammalian cells.[38,119]

Inhibition of re-replication appears to be of central importance for regulation of DNA replication in both prokaryotes and eukaryotes. Multiple mechanisms operate to ensure that re-replication is avoided.[29] In contrast, origin selection or timing determination in eukaryotes appears to be under more plastic regulation.[6] The firing may occur stochastically, with firing probability of each origin being affected by genetic/epigenetic environment or physiological conditions. This sort of plasticity may permit complex cellular systems to respond to varied physiological conditions in a robust and adaptive manner and to complete S phase once committed.

Genomic DNA is very vulnerable during the course of DNA replication, since chromatin proteins are removed and duplex DNA becomes single strand. Stalled replication forks can turn into catastrophic DNA lesions if not corrected immediately. Initial oncogenic stress was reported to reduce the level of nucleotide supply, causing replication stress reactions.[105,106]

Stalled replication forks are quickly recognized in both prokaryotes and eukaryotes. Fork restart by PriA is crucial in prokaryotes,[95] whereas stabilization and protection of stalled forks by replication fork auxiliary factors may be important in eukaryotes to prevent fork collapse or fork inactivation.

The role of membranes in replication and partition remains elusive even after 50 years. However, recent studies suggest the importance of nuclear membranes for regulation of chromosome dynamics including replication, repair, and transcription.[120,121] After all these years, we are still being guided by the great Replicon Theory.

Acknowledgements

I would like to deeply thank Dr. Ken-ichi Arai for leading me to the field of DNA replication 32 years ago and his continuous guidance and support through all the past years. I would also like to thank all my collaborators in and out of my laboratory who contributed to the story I described here and Drs. Keiji Tanaka and Hiromichi Yonekawa for their kind support of our research for the past decade after I joined the current institute. I would like to thank Dr. Dave Gilbert for critical reading of the manuscript and Dr. Yutaka Kanoh for assistance in preparation of figures. Finally, I would like to dedicate this article to my late mother, Yaeko Masai (who passed away on October 23, 2012), who did so much for me and for whom I did so little.

Received 18 January 2013;
Received in revised form 15 March 2013;
Accepted 21 March 2013
Available online 8 April 2013

Keywords:
Replicon Theory;
R1 plasmid;
replication origin;
initiator;
replication timing

References

1. Jacob, F., Brenner, C. & Cuzin, D. (1963). On the regulation of DNA replication in bacteria. *Cold Spring Harbor Quant. Biol.* **28**, 329–348.
2. Kohiyama, M., Cousin, D., Ryter, A. & Jacob, F. (1966). Thermosensitive mutants of *Escherichia coli* K 12. I. Isolation and rapid characterization. *Ann. Inst. Pasteur (Paris)*, **110**, 465–486; (French).
3. Masters, M. & Broda, P. (1971). Evidence for the bidirectional replications of the *Escherichia coli* chromosome. *Nat. New Biol.* **232**, 137–140.
4. Fuller, R. S., Funnell, B. E. & Kornberg, A. (1984). The dnaA protein complex with the *E. coli* chromosomal replication origin (oriC) and other DNA sites. *Cell*, **38**, 889–900.
5. Bell, S. P. & Stillman, B. (1992). ATP-dependent recognition of eukaryotic origins of DNA replication by a multiprotein complex. *Nature*, **357**, 128–134.
6. Masai, H., Matsumoto, S., You, Z., Yoshizawa-Sugata, N. & Oda, M. (2010). Eukaryotic chromosome DNA replication: where, when, and how? *Annu. Rev. Biochem.* **279**, 89–130.
7. Kornberg, A. (2000). Ten commandments: lessons from the enzymology of DNA replication. *J. Bacteriol.* **182**, 3613–3618.
8. Kornberg, A. (1979). The enzymatic replication of DNA. *CRC Crit. Rev. Biochem.* **7**, 23–43.
9. Challberg, M. D. & Kelly, T. J. (1989). Animal virus DNA replication. *Annu. Rev. Biochem.* **58**, 671–717.
10. Fuller, R. S., Kaguni, J. M. & Kornberg, A. (1981). Enzymatic replication of the origin of the *Escherichia coli* chromosome. *Proc. Natl Acad. Sci. USA*, **78**, 7370–7374.
11. Kaguni, J. M. & Kornberg, A. (1984). Replication initiated at the origin (oriC) of the *E. coli* chromosome reconstituted with purified enzymes. *Cell*, **38**, 183–190.
12. Gille, H. & Messer, W. (1991). Localized DNA melting and structural pertubations in the origin of replication, oriC, of *Escherichia coli* in vitro and in vivo. *EMBO J.* **10**, 1579–1584.
13. Yoshikawa, H. & Sueoka, N. (1963). Sequential replication of *Bacillus subtilis* chromosome. I. Comparison of marker frequencies in exponential and stationary growth phases. *Proc. Natl Acad. Sci. USA*, **49**, 559–566.
14. Yasuda, S. & Hirota, Y. (1977). Cloning and mapping of the replication origin of *Escherichia coli*. *Proc. Natl Acad. Sci. USA*, **74**, 5458–5462.
15. Hiraga, S. (1976). Novel F prime factors able to replicate in *Escherichia coli* Hfr strains. *Proc. Natl Acad. Sci. USA*, **73**, 198–202.
16. Miki, T., Hiraga, S., Nagata, T. & Yura, T. (1978). Bacteriophage lambda carrying the *Escherichia coli* chromosomal region of the replication origin. *Proc. Natl Acad. Sci. USA*, **75**, 5099–5103.
17. von Meyenburg, K., Hansen, F. G., Nielsin, L. D. & Riise, E. (1978). Origin of replication, *oriC*, or the *Escherichia coli* chromosome on specialized transducing phages lambda asn. *Mol. Gen. Genet.* **160**, 287–295.
18. Kolter, R. & Helinski, D. R. (1979). Regulation of initiation of DNA replication. *Annu. Rev. Genet.* **13**, 355–391.
19. Nordström, K. (2006). Plasmid R1—replication and its control. *Plasmid*, **55**, 1–26.
20. Kollek, R., Oertel, W. & Goebel, W. (1978). Isolation and characterization of the minimal fragment required for autonomous replication ("basic replicon") of a copy mutant (pKN102) of the antibiotic resistance factor R1. *Mol. Gen. Genet.* **162**, 51–57.
21. Diaz, R., Nordström, K. & Staudenbauer, W. L. (1981). Plasmid R1 DNA replication dependent on protein synthesis in cell-free extracts of *E. coli*. *Nature*, **289**, 326–328.
22. Masai, H., Kaziro, Y. & Arai, K. (1983). Definition of oriR, the minimum DNA segment essential for initiation of R1 plasmid replication in vitro. *Proc. Natl Acad. Sci. USA*, **80**, 6814–6818.
23. Masai, H. & Arai, K. (1987). RepA and DnaA proteins are required for initiation of R1 plasmid replication in vitro and interact with the oriR sequence. *Proc. Natl Acad. Sci. USA*, **84**, 4781–4785.
24. Bramhill, D. & Kornberg, A. (1988). A model for initiation at origins of DNA replication. *Cell*, **54**, 915–918.
25. Masai, H. & Arai, K. (1988). RepA protein- and oriR-dependent initiation of R1 plasmid replication: identification of a rho-dependent transcription terminator required for cis-action of repA protein. *Nucleic Acids Res.* **16**, 6493–6514.
26. Masai, H. & Arai, K. (1988). Book chapter: "R1 plasmid replication *in vitro*: repA- and dnaA-dependent initiation at *oriR*". In *DNA Replication and Mutagenesis* (Moses, R. E. & Summers, W. C., eds), pp. 113–121, American Society for Microbiology, Washington.
27. Blow, J. J. & Laskey, R. A. (1988). A role for the nuclear envelope in controlling DNA replication within the cell cycle. *Nature*, **332**, 546–548.
28. Katayama, T., Ozaki, S., Keyamura, K. & Fujimitsu, K. (2010). Regulation of the replication cycle: conserved and diverse regulatory systems for DnaA and oriC. *Nat. Rev. Microbiol.* **8**, 163–170.
29. Arias, E. E. & Walter, J. C. (2007). Strength in numbers: preventing rereplication via multiple mechanisms in eukaryotic cells. *Genes Dev.* **21**, 497–518.
30. Tomizawa, J.-I., Ohmori, H. & Bird, R. E. (1977). Origin of replication of colicin E1 plasmid DNA. *Proc. Natl Acad. Sci. USA*, **74**, 1865–1869.
31. Kolter, R., Inuzuka, M. & Helinski, D. R. (1978). Trans-complementation-dependent replication of a low molecular weight origin fragment from plasmid R6K. *Cell*, **5**, 1199–1208.
32. Tsurimoto, T. & Matsubara, K. (1981). Purified bacteriophage lambda O protein binds to four repeating sequences at the lambda replication origin. *Nucleic Acids Res.* **9**, 1789–1799.
33. Murotsu, T., Tsutsui, H. & Matsubara, K. (1984). Identification of the minimal essential region for the replication origin of miniF plasmid. *Mol. Gen. Genet.* **196**, 373–378.
34. Sugiura, S., Ohkubo, S. & Yamaguchi, K. (1993). Minimal essential origin of plasmid pSC101 replication: requirement of a region downstream of iterons. *J. Bacteriol.* **175**, 5993–6001.
35. Kittell, B. L. & Helinski, D. R. (1991). Iteron inhibition of plasmid RK2 replication in vitro: evidence for

intermolecular coupling of replication origins as a mechanism for RK2 replication control. *Proc. Natl Acad. Sci. USA*, **88**, 1389–1393.
36. Vashee, S., Cvetic, C., Lu, W., Simancek, P., Kelly, T. J. & Walter, J. C. (2003). Sequence-independent DNA binding and replication initiation by the human origin recognition complex. *Genes Dev.* **17**, 1894–1908.
37. Debatisse, M., Toledo, F. & Anglana, M. (2004). Replication initiation in mammalian cells: changing preferences. *Cell Cycle*, **3**, 19–21.
38. Dellino, G. I., Cittaro, D., Piccioni, R., Luzi, L., Banfi, S., Segalla, S. *et al.* (2012). Genome-wide mapping of human DNA-replication origins: Levels of transcription at ORC1 sites regulate origin selection and replication timing. *Genome Res.* **23**, 1–11.
39. Lucas, I., Chevrier-Miller, M., Sogo, J. M. & Hyrien, O. (2000). Mechanisms ensuring rapid and complete DNA replication despite random initiation in *Xenopus* early embryos. *J. Mol. Biol.* **296**, 769–786.
40. Patel, P. K., Arcangioli, B., Baker, S. P., Bensimon, A. & Rhind, N. (2006). DNA replication origins fire stochastically in fission yeast. *Mol. Biol. Cell*, **17**, 308–316.
41. Herrick, J., Jun, S., Bechhoefer, J. & Bensimon, A. (2002). Kinetic model of DNA replication in eukaryotic organisms. *J. Mol. Biol.* **320**, 741–750.
42. Bramhill, D. & Kornberg, A. (1988). Duplex opening by dnaA protein at novel sequences in initiation of replication at the origin of the *E. coli* chromosome. *Cell*, **52**, 743–755.
43. Matsunaga, F., Forterre, P., Ishino, Y. & Myllykallio, H. (2001). In vivo interactions of archaeal Cdc6/Orc1 and minichromosome maintenance proteins with the replication origin. *Proc. Natl Acad. Sci. USA*, **98**, 11152–11157.
44. Gaudier, M., Schuwirth, B. S., Westcott, S. L. & Wigley, D. B. (2007). Structural basis of DNA replication origin recognition by an ORC protein. *Science*, **317**, 1213–1216.
45. Samson, R. Y., Xu, Y., Gadelha, C., Stone, T. A., Faqiri, J. N., Li, D. *et al.* (2013). Specificity and function of archaeal DNA replication initiator proteins. *Cell Rep.* **3**, 485–496.
46. Tanaka, T. & Nasmyth, K. (1998). Association of RPA with chromosomal replication origins requires an Mcm protein, and is regulated by Rad53, and cyclin- and Dbf4-dependent kinases. *EMBO J.* **17**, 5182–5191.
47. Remus, D., Beuron, F., Tolun, G., Griffith, J. D., Morris, E. P. & Diffley, J. F. (2009). Concerted loading of Mcm2-7 double hexamers around DNA during DNA replication origin licensing. *Cell*, **139**, 719–730.
48. Evrin, C., Clarke, P., Zech, J., Lurz, R., Sun, J., Uhle, S. *et al.* (2009). Double-hexameric MCM2-7 complex is loaded onto origin DNA during licensing of eukaryotic DNA replication. *Proc. Natl Acad. Sci. USA*, **106**, 20240–20245.
49. Moyer, S. E., Lewis, P. W. & Botchan, M. R. (2006). Isolation of the Cdc45/Mcm2-7/GINS (CMG) complex, a candidate for the eukaryotic DNA replication fork helicase. *Proc. Natl Acad. Sci. USA*, **103**, 10236–10241.
50. Fu, Y. V., Yardimci, H., Long, D. T., Ho, T. V., Guainazzi, A., Bermudez, V. P. *et al.* (2011). Selective bypass of a lagging strand roadblock by the eukaryotic replicative DNA helicase. *Cell*, **146**, 931–941.
51. Heller, R. C., Kang, S., Lam, W. M., Chen, S., Chan, C. S. & Bell, S. P. (2011). Eukaryotic origin-dependent DNA replication in vitro reveals sequential action of DDK and S-CDK kinases. *Cell*, **146**, 80–91.
52. Woodward, A. M., Göhler, T., Luciani, M. G., Oehlmann, M., Ge, X., Gartner, A. *et al.* (2006). Excess Mcm2-7 license dormant origins of replication that can be used under conditions of replicative stress. *J. Cell Biol.* **173**, 673–683.
53. Ibarra, A., Schwob, E. & Méndez, J. (2008). Excess MCM proteins protect human cells from replicative stress by licensing backup origins of replication. *Proc. Natl Acad. Sci. USA*, **105**, 8956–8961.
54. Czajkowsky, D. M., Liu, J., Hamlin, J. L. & Shao, Z. (2008). DNA combing reveals intrinsic temporal disorder in the replication of yeast chromosome VI. *J. Mol. Biol.* **375**, 12–19.
55. Shirahige, K., Hori, Y., Shiraishi, K., Yamashita, M., Takahashi, K., Obuse, C. *et al.* (1998). Regulation of DNA-replication origins during cell-cycle progression. *Nature*, **395**, 618–621.
56. Santocanale, C. & Diffley, J. F. (1998). A Mec1- and Rad53-dependent checkpoint controls late-firing origins of DNA replication. *Nature*, **395**, 615–618.
57. Zegerman, P. & Diffley, J. F. (2010). Checkpoint-dependent inhibition of DNA replication initiation by Sld3 and Dbf4 phosphorylation. *Nature*, **467**, 474–478.
58. Lopez-Mosqueda, J., Maas, N. L., Jonsson, Z. O., Defazio-Eli, L. G., Wohlschlegel, J. & Toczyski, D. P. (2010). Damage-induced phosphorylation of Sld3 is important to block late origin firing. *Nature*, **467**, 479–483.
59. Wu, P. Y. & Nurse, P. (2009). Establishing the program of origin firing during S phase in fission yeast. *Cell*, **136**, 852–864.
60. Tanaka, S., Nakato, R., Katou, Y., Shirahige, K. & Araki, H. (2011). Origin association of Sld3, Sld7, and Cdc45 proteins is a key step for determination of origin-firing timing. *Curr. Biol.* **21**, 2055–2063.
61. Mantiero, D., Mackenzie, A., Donaldson, A. & Zegerman, P. (2011). Limiting replication initiation factors execute the temporal programme of origin firing in budding yeast. *EMBO J.* **30**, 4805–4814.
62. Knott, S. R., Viggiani, C. J., Tavaré, S. & Aparicio, O. M. (2009). Genome-wide replication profiles indicate an expansive role for Rpd3L in regulating replication initiation timing or efficiency, and reveal genomic loci of Rpd3 function in *Saccharomyces cerevisiae*. *Genes Dev.* **23**, 1077–1090.
63. Miotto, B. & Struhl, K. (2008). HBO1 histone acetylase is a coactivator of the replication licensing factor Cdt1. *Genes Dev.* **22**, 2633–2638.
64. Knott, S. R., Peace, J. M., Ostrow, A. Z., Gan, Y., Rex, A. E., Viggiani, C. J. *et al.* (2012). Forkhead transcription factors establish origin timing and long-range clustering in *S. cerevisiae*. *Cell*, **148**, 99–111.
65. Yompakdee, C. & Huberman, J. A. (2004). Enforcement of late replication origin firing by clusters of

short G-rich DNA sequences. *J. Biol. Chem.* **279**, 42337–42344.
66. Tazumi, A., Fukuuram, M., Nakato, R., Kishimoto, A., Takenaka, T., Ogawa, S. *et al.* (2012). Telomere-binding protein Taz1 controls global replication timing through its localization near late replication origins in fission yeast. *Genes Dev.* **26**, 2050–2062.
67. Masai, H. & Arai, K. (2002). Cdc7 kinase complex: a key regulator in the initiation of DNA replication. *J. Cell. Physiol.* **190**, 287–296.
68. Labib, K. (2010). How do Cdc7 and cyclin-dependent kinases trigger the initiation of chromosome replication in eukaryotic cells? *Genes Dev.* **24**, 1208–1219.
69. Hardy, C. F., Dryga, O., Seematter, S., Pahl, P. M. & Sclafani, R. A. (1997). mcm5/cdc46-bob1 bypasses the requirement for the S phase activator Cdc7p. *Proc. Natl Acad. Sci. USA*, **94**, 3151–3155.
70. Hoang, M. L., Leon, R. P., Pessoa-Brandao, L., Hunt, S., Raghuraman, M. K., Fangman, W. L. *et al.* (2007). Structural changes in Mcm5 protein bypass Cdc7-Dbf4 function and reduce replication origin efficiency in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **27**, 7594–7602.
71. Sheu, Y. J. & Stillman, B. (2010). The Dbf4-Cdc7 kinase promotes S phase by alleviating an inhibitory activity in Mcm4. *Nature*, **463**, 113–117.
72. Hayano, M., Kanoh, Y., Matsumoto, S. & Masai, H. (2011). Mrc1 marks early-firing origins and coordinates timing and efficiency of initiation in fission yeast. *Mol. Cell. Biol.* **31**, 2380–2391.
73. Matsumoto, S., Hayano, M., Kanoh, Y. & Masai, H. (2011). Multiple pathways can bypass the essential role of fission yeast Hsk1 kinase in DNA replication initiation. *J. Cell Biol.* **195**, 387–401.
74. Hayano, M., Kanoh, Y., Matsumoto, S., Renard-Guillet, C., Shirahige, K. & Masai, H. (2012). Rif1 is a global regulator of timing of replication origin firing in fission yeast. *Genes Dev.* **26**, 137–150.
75. Huberman, J. A. & Riggs, A. D. (1968). On the mechanism of DNA replication in mammalian chromosomes. *J. Mol. Biol.* **32**, 327–341.
76. Nakamura, H., Morita, T. & Sato, C. (1986). Structural organizations of replicon domains during DNA synthetic phase in the mammalian nucleus. *Exp. Cell Res.* **165**, 291–297.
77. Hiratani, I., Ryba, T., Itoh, M., Yokochi, T., Schwaiger, M., Chang, C. W. *et al.* (2008). Global reorganization of replication domains during embryonic stem cell differentiation. *PLoS Biol.* **6**, e245.
78. Weddington, N., Stuy, A., Hiratani, I., Ryba, T., Yokochi, T. & Gilbert, D. M. (2008). ReplicationDomain: a visualization tool and comparative database for genome-wide replication timing data. *BMC Bioinformatics*, **9**, 530.
79. Dimitrova, D. S. & Gilbert, D. M. (1999). The spatial position and replication timing of chromosomal domains are both established in early G1 phase. *Mol. Cell*, **4**, 983–993.
80. Yamazaki, S., Ishii, A., Kanoh, Y., Oda, M., Nishito, Y. & Masai, H. (2012). Rif1 regulates the replication timing domains on the human genome. *EMBO J.* **31**, 3667–3677.
81. Cornacchia, D., Dileep, V., Quivy, J. P., Foti, R., Tili, F., Santarella-Mellwig, R. *et al.* (2012). Mouse Rif1 is a key regulator of the replication-timing programme in mammalian cells. *EMBO J.* **31**, 3678–3690.
82. Heun, P., Laroche, T., Raghuraman, M. K. & Gasser, S. M. (2001). The positioning and dynamics of origins of replication in the budding yeast nucleus. *J. Cell Biol.* **152**, 385–400.
83. Park, S., Patterson, E. E., Cobb, J., Audhya, A., Gartenberg, M. R. & Fox, C. A. (2011). Palmitoylation controls the dynamics of budding-yeast heterochromatin via the telomere-binding protein Rif1. *Proc. Natl Acad. Sci. USA*, **108**, 14572–14577.
84. Vaziri, C., Saxena, S., Jeon, Y., Lee, C., Murata, K., Machida, Y. *et al.* (2003). A p53-dependent checkpoint pathway prevents rereplication. *Mol. Cell*, **11**, 997–1008.
85. Tatsumi, Y., Sugimoto, N., Yugawa, T., Narisawa-Saito, M., Kiyono, T. & Fujita, M. (2006). Deregulation of Cdt1 induces chromosomal damage without rereplication and leads to chromosomal instability. *J. Cell Sci.* **119**, 3128–3140.
86. Davidson, I. F., Li, A. & Blow, J. J. (2006). Deregulated replication licensing causes DNA fragmentation consistent with head-to-tail fork collision. *Mol. Cell*, **24**, 433–443.
87. Rhind, N., Yang, S. C.-H. & Bechhoefer, J. (2010). Reconciling stochastic origin firing with defined replication timing. *Chromosome Res.* **18**, 35–43.
88. Schekman, R., Weiner, J. H., Weiner, A. & Kornberg, A. (1975). Ten proteins required for conversion of phiX174 single-stranded DNA to duplex form in vitro. Resolution and reconstitution. *J. Biol. Chem.* **250**, 5859–5865.
89. Masai, H., Bond, M. W. & Arai, K. (1986). Cloning of the *Escherichia coli* gene for primosomal protein i: the relationship to dnaT, essential for chromosomal DNA replication. *Proc. Natl Acad. Sci. USA*, **83**, 1256–1260.
90. Lee, E. H., Masai, H., Allen, G. C., Jr. & Kornberg, A. (1990). The priA gene encoding the primosomal replicative n' protein of *Escherichia coli*. *Proc. Natl Acad. Sci. USA*, **87**, 4620–4624.
91. Nurse, P., DiGate, R. J., Zavitz, K. H. & Marians, K. J. (1990). Molecular cloning and DNA sequence analysis of *Escherichia coli* priA, the gene encoding the primosomal protein replication factor Y. *Proc. Natl Acad. Sci. USA*, **87**, 4615–4619.
92. Masai, H., Asai, T., Kubota, Y., Arai, K. & Kogoma, T. (1994). *Escherichia coli* PriA protein is essential for inducible and constitutive stable DNA replication. *EMBO J.* **13**, 5338–5345.
93. Lee, E. H. & Kornberg, A. (1991). Replication deficiencies in priA mutants of *Escherichia coli* lacking the primosomal replication n' protein. *Proc. Natl Acad. Sci. USA*, **88**, 3029–3032.
94. Nurse, P., Zavitz, K. H. & Marians, K. J. (1991). Inactivation of the *Escherichia coli* priA DNA replication protein induces the SOS response. *J. Bacteriol.* **173**, 6686–6693.
95. Sandler, S. J. & Marians, K. J. (2000). Role of PriA in replication fork reactivation in *Escherichia coli*. *J. Bacteriol.* **182**, 9–13.
96. McGlynn, P., Al-Deib, A. A., Liu, J., Marians, K. J. & Lloyd, R. G. (1997). The DNA replication protein PriA

and the recombination protein RecG bind D-loops. *J. Mol. Biol.* **270**, 212–221.
97. Kogoma, T., Cadwell, G. W., Barnard, K. G. & Asai, T. (1996). The DNA replication priming protein, PriA, is required for homologous recombination and double-strand break repair. *J. Bacteriol.* **178**, 1258–1264.
98. Heller, R. C. & Marians, K. J. (2005). The disposition of nascent strands at stalled replication forks dictates the pathway of replisome loading during restart. *Mol. Cell*, **17**, 733–743.
99. Mizukoshi, T., Tanaka, T., Arai, K., Kohda, D. & Masai, H. (2003). A critical role of the 3′ terminus of nascent DNA chains in recognition of stalled replication forks. *J. Biol. Chem.* **278**, 42234–42239.
100. Sasaki, K., Ose, T., Okamoto, N., Maenaka, K., Tanaka, T., Masai, H. *et al.* (2007). Structural basis of the 3′-end recognition of a leading strand in stalled replication forks by PriA. *EMBO J.* **26**, 2584–2593.
101. Masai, H., Tanaka, T. & Kohda, D. (2010). Stalled replication forks: making ends meet for recognition and stabilization. *BioEssays*, **32**, 687–697.
102. De Piccoli, G., Katou, Y., Itoh, T., Nakato, R., Shirahige, K. & Labib, K. (2012). Replisome stability at defective DNA replication forks is independent of S phase checkpoint kinases. *Mol. Cell*, **45**, 696–704.
103. Gambus, A., Jones, R. C., Sanchez-Diaz, A., Kanemaki, M., van Deursen, F., Edmondson, R. D. & Labib, K. (2006). GINS maintains association of Cdc45 with MCM in replisome progression complexes at eukaryotic DNA replication forks. *Nat. Cell Biol.*, 358–366.
104. Blow, J. J., Ge, X. Q. & Jackson, D. A. (2011). How dormant origins promote complete genome replication. *Trends Biochem. Sci.* **36**, 405–414.
105. Bester, A. C., Roniger, M., Oren, Y. S., Im, M. M., Sarni, D., Chaoat, M. *et al.* (2011). Nucleotide deficiency promotes genomic instability in early stages of cancer development. *Cell*, **145**, 435–446.
106. Bartkova, J., Horejsí, Z., Koed, K., Krämer, A., Tort, F., Zieger, K. *et al.* (2005). DNA damage response as a candidate anti-cancer barrier in early human tumorigenesis. *Nature*, **434**, 864–870.
107. Gorgoulis, V. G., Vassiliou, L. V., Karakaidos, P., Zacharatos, P., Kotsinas, A., Liloglou, T. *et al.* (2005). Activation of the DNA damage checkpoint and genomic instability in human precancerous lesions. *Nature*, **434**, 907–913.
108. Iraqui, I., Chekkal, Y., Jmari, N., Pietrobon, V., Fréon, K., Costes, A. & Lambert, S. A. (2012). Recovery of arrested replication forks by homologous recombination is error-prone. *PLoS Genet*, **8**, e1002976.
109. Mizuno, K., Miyabe, I., Schalbetter, S. A., Carr, A. M. & Murray, J. M. (2013). Recombination-restarted replication makes inverted chromosome fusions at inverted repeats. *Nature*, **493**, 246–249.
110. McKinnon, P. J. & Caldecott, K. W. (2007). DNA strand break repair and human genetic disease. *Annu. Rev. Genomics Hum. Genet.* **8**, 37–55.
111. Monnat, R. J., Jr. (2010). Human RECQ helicases: roles in DNA metabolism, mutagenesis and cancer biology. *Semin. Cancer Biol.* **20**, 329–339.
112. Kornberg, A. & Baker, T. A. (1992). *DNA Replication*, 2nd edit. W. H. Freeman & Co, New York, NY.
113. Gilbert, D. M. (2001). Making sense of eukaryotic DNA replication origins. *Science*, **294**, 96–100.
114. Cayrou, C., Coulombe, P. & Méchali, M. (2010). Programming DNA replication origins and chromosome organization. *Chromosome Res.* **18**, 137–145.
115. Gilbert, D. M. (2010). Evaluating genome-scale approaches to eukaryotic DNA replication. *Nat. Rev. Genet.* **11**, 673–684.
116. Cayrou, C., Coulombe, P., Vigneron, A., Stanojcic, S., Ganier, O., Peiffer, I. *et al.* (2011). Genome-scale analysis of metazoan replication origins reveals their organization in specific but flexible sites defined by conserved features. *Genome Res.* **21**, 1438–1449.
117. Xu, J., Yanagisawa, Y., Tsankov, A. M., Hart, C., Aoki, K., Kommajosyula, N. *et al.* (2012). Genome-wide identification and characterization of replication origins by deep sequencing. *Genome Biol.* **13**, R27.
118. Sekimizu, K., Bramhill, D. & Kornberg, A. (1987). ATP activates dnaA protein in initiating replication of plasmids bearing the origin of the *E. coli* chromosome. *Cell*, **50**, 259–265.
119. MacAlpine, H. K., Gordân, R., Powell, S. K., Hartemink, A. J. & MacAlpine, D. M. (2010). Drosophila ORC localizes to open chromatin and marks sites of cohesin complex loading. *Genome Res.* **20**, 201–211.
120. Bermejo, R., Capra, T., Jossen, R., Colosio, A., Frattini, C., Carotenuto, W. *et al.* (2011). The replication checkpoint protects fork stability by releasing transcribed genes from nuclear pores. *Cell*, **46**, 233–246.
121. Nagai, S., Heun, P. & Gasser, S. M. (2010). Roles for nuclear organization in the maintenance of genome stability. *Epigenomics*, **2**, 289–305.